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Incidence of Human Calicivirus and Rotavirus Infection in Patients With Gastroenteritis in South Africa

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Human caliciviruses (HuCVs) are reportedly responsible for 2.5–4% of nonbacterial sporadic gastroenteritis. The incidence of HuCV infection in South Africa is unknown. Stool specimens from 1,296 South African patients with sporadic gastroenteritis were screened for the presence of HuCVs using electron microscopy, recombinant enzyme immunoassays for Norwalk (NV) and Mexican (MX) viruses, and the reverse transcriptase-polymerase chain reaction (RT-PCR). RT-PCR products were sequenced to ascertain which HuCV genogroups were present. HuCVs were detected in 43/1,296 (3.3%) specimens examined, with RT-PCR proving to be the most sensitive detection method. Genetic analysis of the isolates indicated that 81% were Snow Mountain Agent, or MX-like; 8% were NV-like; and 11% were HuCV/Sapporo-like. This study indicates that a combination of assays is needed for the accurate detection of HuCVs. Comparative data on hospitalised patients showed that the incidence of rotavirus infection was approximately ten times greater than that of HuCV infection. ***J Med Virol 51:290–296, 1997.***

KEY WORDS: human calicivirus; rotavirus; gastroenteritis

INTRODUCTION

Caliciviruses are a cause of many diseases in both humans and animals [Norcott et al., 1994]. Human caliciviruses (HuCVs) are mainly associated with sporadic acute gastroenteritis in young children and community outbreaks involving children and adults [Cubitt, 1989]. HuCVs reportedly account for 2.5–4% of non-bacterial sporadic gastroenteritis in some parts of the world [Dinulos and Matson, 1994; Green et al., 1995a]. However, the true incidence of HuCV-related infections is unknown as many cases of viral gastroenteritis are unreported or a definitive diagnosis is not made [Cubitt, 1987; Green et al., 1995a]. As HuCVs have to date not been cultivated in cell culture systems, the diagnosis of and research on HuCV infection has, until recently, been limited to the use of techniques such as electron microscopy (EM), immune electron microscopy (IEM), or enzyme immunoassays (EIAs) using reagents from volunteer studies [Kapikian, 1994]. The cloning and characterisation of the Norwalk virus (NV) genome and the subsequent expression of recombinant NV (rNV) antigen in baculovirus enabled the development of specific and sensitive EIAs for the detection of NV antigen and antibodies [Graham et al., 1994]. Subsequently, the viral capsid gene of another strain of HuCV, HuCV/MX/89/Mexico (MX), has been cloned, sequenced, and expressed in baculovirus [Jiang et al., 1995a,b]. The latter virus shares a higher percentage of amino acid identity (91%) with Snow Mountain Agent (SMA) than with NV (60%) in the RNA-dependent RNA polymerase region of the genome [Jiang et al., 1995a]. Although a low level of cross-reaction between NV and SMA has been demonstrated in the rNV antibody assays [Treanor et al., 1993; Jiang et al., 1995b], no cross-reactions between NV and MX have been detected in the recombinant antigen assays [Jiang et al., 1995b]. The availability of nucleic acid sequence information of the NV genome [Jiang et al., 1990], the Southampton virus (SHV) [Lambden et al., 1993], the Bristol virus [Green et al., 1994], and many other strains of HuCV has facilitated the development of reverse transcriptase-polymerase chain reaction (RT-PCR) assays for the detection and molecular characterisation of these viruses [Jiang et al., 1992; Green et al., 1993; Cubitt et

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al., 1994; Lew et al., 1994]. On the basis of sequence homologies HuCVs can be divided into three genogroups: NV-like, SMA-like, and HuCV/Sapporo-like [Jiang et al., 1995c; Liu et al., 1995; Matson et al., 1995]. The relative importance of each genogroup in causing human disease is unknown, although currently the SMA genogroup appears to predominate [Jiang et al., 1995c,d].

The occurrence of HuCV-associated epidemic gastroenteritis in South Africa has been documented and the viruses partially characterised [Taylor et al., 1993; Steele et al., 1995a; Wolfaardt et al., 1995]. Although HuCVs have been identified in association with paediatric gastroenteritis [Tiemessen et al., 1989; Wolfaardt et al., 1995], little data are available on the role of HuCVs in sporadic and paediatric gastroenteritis. The present study reports on the incidence of HuCV infection associated with sporadic gastroenteritis in selected areas of South Africa.

MATERIALS AND METHODS

Clinical Specimens

A total of 1,296 faecal specimens from sporadic cases of gastroenteritis, submitted to selected laboratories in different geographical regions of South Africa for routine diagnostic procedures between October 1991 and October 1995, were included in this study. Specimens were from Durban (n = 14), Stellenbosch (n = 66), Soweto (n = 127), and Pretoria (n = 1,091), and, where available, data indicating age, sex, and ethnic origin of patients were recorded. One hundred and thirty-two of these specimens were from non-hospitalised gastroenteritis cases in a low socioeconomic community. The remaining specimens were from hospitalised gastroenteritis patients. The latter patients were from both lower and higher socioeconomic communities, and it is not known whether or not the gastroenteritis was nosocomially or community acquired. The HIV and immune status of the individual patients was not established. Stool specimens were stored at 4°C until tested.

Electron Microscopy

Faecal specimens were prepared for direct EM by mixing a drop of stool with 20–50 μl of 2% phosphotungstic acid (pH 6.8) and placing the suspension on a 400-mesh Formvar carbon-coated grid. The grid was air-dried and examined in a Philips (Eindhoven, Holland) 300 electron microscope.

Enzyme Immunoassays for the Detection of NV and MX Antigens

Stool samples were analysed for the presence of NV and MX antigens using the methods described by Graham and coworkers [1994] and Jiang and colleagues [1995b,d], respectively. These assays use rabbit antisera raised against the respective recombinant antigens, namely rNV or rMX, as the coating antibody and guinea pig antisera raised against rNV or rMX as the detector antibody. In both assays, two wells were used for each sample, one coated with rabbit preimmune serum and the other with rabbit hyperimmune serum. The reaction was visualised using horseradish peroxidase (HRP)-conjugated goat antiguinea pig serum (Hyclone, Logan, UT) and 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB) (Kirkegaard & Perry Laboratories, Gaithersburg, MD) as substrate. Specimens were considered to be positive if an optical density (OD) ⩾1 together with a P/N >2 (OD value with rabbit hyperimmune serum/OD with rabbit preimmunisation serum) was demonstrated. NV- and MX-positive stool specimens were included as controls in each plate.

Assays for the Detection of Rotavirus

As stool specimens used in this investigation had been submitted for routine examination for gastroenteritis viruses, all 1,296 stools were assayed for the presence of rotavirus by either latex agglutination (LA) assay (Murex Rotavirus Latex Test; Murex Diagnostics, Dartford, UK), EIA (Rotaclone; Cambridge Biotech, Worcester, MA), and/or direct EM.

RNA Extraction and RT-PCR Amplification

Viral RNA was extracted from 10% suspensions of stool specimens in distilled water using a polyethylene glycol-cetyltrimethyl ammonium bromide method [Jiang et al., 1992]. The extracted RNA was resuspended in a final volume of 20 μl water.

Oligonucleotide primers were synthesised and purified by The Midland Certified Reagent Company (Midland, TX). Primer pairs NV36/NV35 [Wang et al., 1994] and NV36/NV51 [Moe et al., 1994] were used for screening of the stool specimens. Primer pairs NV36/p101 [Jiang et al., 1995a] and NV36/M313 were used when HuCVs were detected by methods other than the initial RT-PCR screening. Primer M313 (5′-GTG TGA ACG GTC TCC ACT TGG-3′) was derived from the sequence of Sapporo/82 in the region of the YGDD motif of the RNA polymerase gene. The RT-PCR amplification procedures and conditions used were as described previously [Wolfaardt et al., 1995].

Sequencing of PCR Products and Analysis of Sequences

RT-PCR products were sequenced directly using the Sequenase PCR Product Sequencing Kit (US Biochemical Corp., Cleveland, OH). The sequencing reactions were run on an 8% polyacrylamide-6 M urea gel in 1 × TBE buffer. Gels were vacuum-dried and exposed to X-ray film at room temperature for 12 hr.

Sequences of the amplified products were analysed using the CLUSTAL program of the PC/GENE software, version 6.8 (IntelliGenetics, Mountain View, CA). The following sequences were used for the comparisons: HuCV/NV/8FIIa/76/US (NV) [Jiang et al., 1990], HuCV/SMA/79/US (SMA) [Wang et al., 1994], HuCV/MX/89/Mexico (MX) [Jiang et al., 1995a], and HuCV/Sapporo/82/Japan [Matson et al., 1995].

RESULTS

Clinical Specimens

Of the 1,296 faecal specimens analysed, 846 (73%) were from patients younger than 24 months and 79 (5%) from patients older than 10 years of age, with a female to male ratio of 1:1.2. The ethnic origins of the

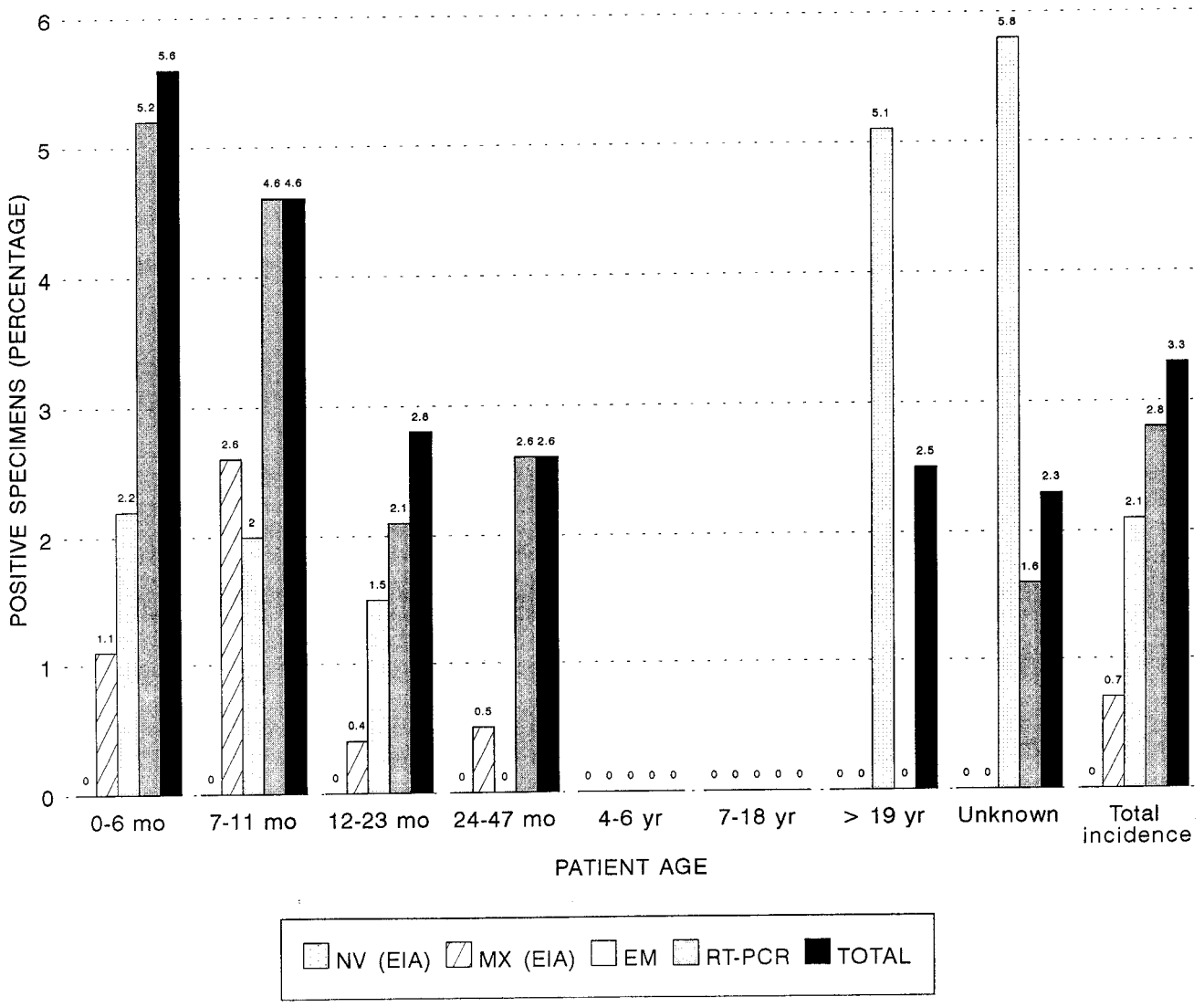

Fig. 1. Incidence of human caliciviruses in stool specimens of patients with sporadic gastroenteritis. NV, Norwalk virus; MX, Mexican virus; EIA, enzyme immunoassay; EM, electron microscopy; RT-PCR, reverse transcriptase-polymerase chain reaction; mo, months; yr, years.

patients were as follows: European (white Caucasian) (51%), Black African (41%), Coloured (mixed descent) (7%), and Asian (1%).

Comparison of the Methods of HuCV Detection

Comparative data on the detection of HuCVs in stool specimens by EM, EIA, and RT-PCR are shown in Figure 1. HuCVs were demonstrated to be either morphologically typical caliciviruses (n = 4) or small round-structured viruses (SRSVs) (n = 8) in 12/560 (2.1%) specimens examined by EM. HuCVs were visualised predominantly in specimens from children of European ethnic origin less than 1 year of age. MX antigen was shown to be present, by EIA, in nine (0.7%) specimens, while NV antigen was not detected in any of the specimens tested. MX antigen was found predominantly in children less than 1 year of age, with infection still detectable up to the age of 4 years. HuCVs were detected in 36 (2.8%) of the specimens by RT-PCR (Fig. 1; Table I). Of the HuCVs detected by RT-PCR, 21 (3.8%) were detected in the 560 specimens examined by EM. However, in six of the specimens where HuCVs with SRSV-like morphology were detected by EM, no HuCVs could be detected by RT-PCR. In the nine specimens where MX antigen was detected by rMX EIA, HuCVs were also demonstrated by RT-PCR. In total, 43 (3.3%) of the patients were shown to harbour HuCV infection. Infection was found to be more prominent in children less than 1 year of age and of African ethnic origin, although children up to 4 years of age and adults were also shown to be infected.

HuCV Genogroups

Partial sequence analysis of the RNA-dependent RNA polymerase region indicated that of the 36 HuCVs detected by RT-PCR, 29 (81%) were SMA- or MX-like, three (8%) were NV-like, and four were HuCV/Sapporo-

TABLE I. Human Calicivirus Genogroups Detected in Different Age Groups

	Number of positive specimens/number of specimens tested (%)		
Age	NV-like	MX-like	Sapporo-like
0–6 mo	1/270 (0.4%)	10/270 (3.7%)	3/270 (1.1%)
7–11 mo	0/151 (0)	6/151 (4%)	1/151 (0.6%)
12–23 mo	2/281 (0.7%)	4/281 (1.4%)	0/281 (0)
24–47 mo	0/190 (0)	5/190 (2.6%)	0/190 (0)
4–6 yr	0/55 (0)	0/55 (0)	0/55 (0)
7–18 yr	0/13 (0)	0/13 (0)	0/13 (0)
>19 yr	0/79 (0)	0/79 (0)	0/79 (0)
Unknown	0/157 (0)	4/157 (2.5%)	0/157 (0)
Total	3/1296 (0.2%)	29/1296 (2.2%)	4/1296 (0.3%)

NV, Norwalk virus
MX, Mexico virus

like (11%) (Table I). The latter four isolates were shown, by EM, to be morphologically typical caliciviruses. The HuCVs detected in the eight specimens by rMX EIA were found to group within the SMA- or MX-like HuCVs. No pattern was observed in the distribution of genogroups with regard to the geographical location or ethnic origin of the patients.

Incidence of Rotavirus Infection

The incidence of rotavirus infection, in relation to HuCV infection, in both hospitalised and nonhospitalised patients is summarised in Figure 2. From Figure 2 it is evident that in hospitalised patients, and for most age groups, the incidence of rotavirus infection was approximately ten times greater than that of HuCV infection. Of note is the relatively high incidence of rotavirus infection in the 4–6-year-old age group compared to the total absence of HuCV infection in this group of patients. In comparison, the overall incidence of rotavirus infection in the nonhospitalised group (10.6%) was of the same order as that of HuCV infection (9.8%), with infections being detected predominantly in children less than 2 years of age.

Incidence of Dual Infections

Dual rotavirus and HuCV infections were noted in four patients, all children less than 1 year of age, three of whom were hospitalised. Concomitant HuCV infections with adenovirus types 40 and 41 (Ad40/41) (n = 1), adenovirus (not Ad40/41) (n = 1), astrovirus (n = 2), and *Giardia lamblia* (n = 1) were also evident.

DISCUSSION

The development and availability of EIAs for the detection of specific HuCVs [Graham et al., 1994; Jiang et al., 1995b,c,d] has facilitated large-scale epidemiological studies to determine the incidence of HuCV infection in communities [Numata et al., 1994; Steele et al., 1995b; Jiang et al., 1995a; Cubitt and Jiang, 1996]. In addition, the use of RT-PCR has enabled the detection and characterisation of HuCVs in specimens where conventional techniques have failed [Herwaldt et al., 1994; Jiang et al., 1995a,c; Wolfaardt et al., 1995]. In the survey of 1,296 stool specimens from South African patients with gastroenteritis and using a combination of EM, EIA, and RT-PCR, HuCVs were demonstrated in 43 (3.3%) specimens, predominantly in specimens from children less than 4 years of age. RT-PCR, using four different sets of primers either singly or in combination, proved to be the most sensitive method for the detection of HuCVs, detecting viral nucleic acid in 36 (2.8%) of the specimens tested. However, RT-PCR failed to detect viral nucleic acid, and neither NV nor MX antigen was detected by EIA in six specimens where HuCVs were demonstrated by direct EM. This may be ascribed to the considerable nucleotide sequence diversity recorded among the HuCVs [Green et al., 1994; Wang et al., 1994; Norcott et al., 1994]. The primers selected for the detection of HuCV in clinical and environmental specimens are therefore an important factor in the sensitivity of the test [Green et al., 1995a,b]. HuCVs were demonstrated by EM in only 12/560 (2.1%) specimens examined. Although EM has low sensitivity for the detection of HuCVs [Green et al., 1995a], it is a useful "catchall technique" in which all viruses present in sufficient numbers can be detected [Monroe et al., 1991]. The recombinant EIAs for the detection of NV and MX antigens proved to be less sensitive than both RT-PCR and EM, detecting MX antigen in only nine (0.7%) and NV antigen in none of the stools screened. These recombinant assays have been shown to be highly specific for NV [Graham et al., 1994] and MX [Jiang et al., 1995b,c], with the rMX assay detecting viruses showing a high percentage (>83%) amino acid sequence identity with SMA and MX viruses [Jiang et al., 1995d]. Consequently, any antigenic variants are less likely to be detected by these assays. This is emphasised in this investigation, where MX-like isolates detected by the rMX antigen assay were found to have a higher percentage (>96%) amino acid sequence identity with MX than isolates which were not detected by the rMX EIA. These data show that a combination of different assays may be required for an accurate diagnosis of HuCV infection.

In the United Kingdom, different serotypes of HuCV were found to be associated with sporadic and epidemic gastroenteritis and to circulate at different time intervals [Lewis et al., 1993]. In this investigation, no clear seasonal, ethnic, or geographical pattern of HuCV infection was noted. Amongst the specimens investi-

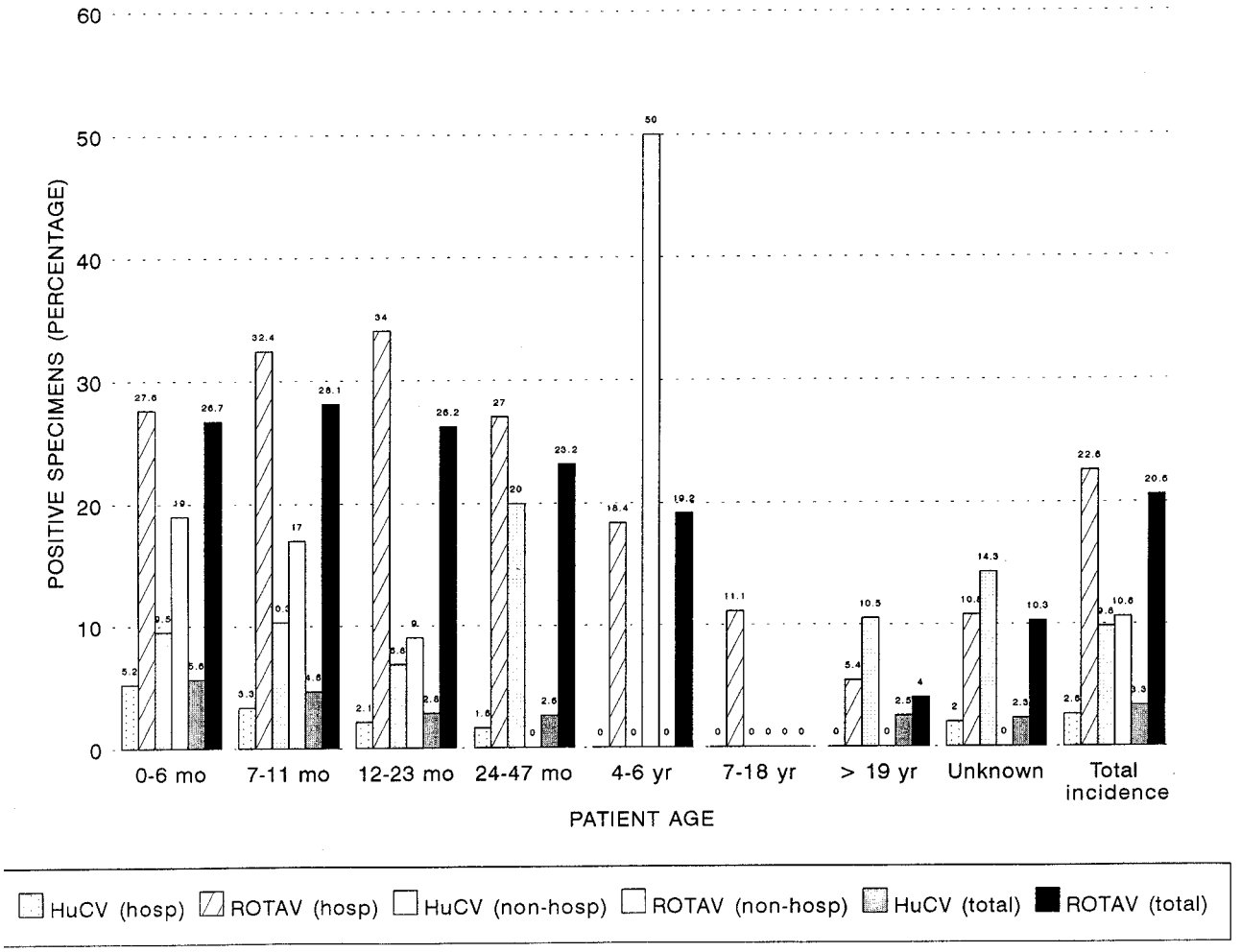

Fig. 2. Incidence of human calicivirus (HuCV) and rotavirus (ROTAV) infection in hospitalised and non-hospitalised patients with gastroenteritis. mo, months; yr, years; hosp, hospitalised; non-hosp, nonhospitalised.

gated, only three NV-like viruses were detected, all from paediatric patients from different geographical regions and time intervals. Although a recent seroepidemiological study indicated that the age of acquisition of NV antibody amongst urban South Africans was early, i.e., in the second year of life (Taylor et al., 1996), these data suggest that NV-like viruses are not an important cause of sporadic paediatric gastroenteritis in the communities investigated. NV infection, however, has been demonstrated by rNV EIA in 1.8% of hospitalised children in a different geographical region of South Africa [Steele et al., 1995b]. NV-like viruses have also been associated with two epidemics of adult gastroenteritis from two distinct geographical regions, in 1989 and 1991 [Wolfaardt et al., 1995], emphasising that regional differences may play an important role in the incidence of HuCV infection in South Africa. SMA- or MX-like viruses were found to be the most common HuCV identified, specifically in children less than 2 years of age, suggesting that SMA- or MX-like viruses potentially play a more important role than NV in paediatric gastroenteritis. This view is supported by serological data from London, UK, where MX infections appear to occur earlier in life while NV infection occurs later, predominantly in older children and adults [Parker et al., 1995]. An SMA-like virus was found to be associated with an outbreak of adult and paediatric gastroenteritis in a different geographical region during the same study period (1994) [Steele et al., 1995a], suggesting that currently SMA- or MX-like infections may be more widespread than NV-like infection.

In this investigation, the overall incidence of HuCV infection was found to be higher in patients of black African origin, while the majority of the EM-positive specimens were from patients of European ethnic origin. This phenomenon may be ascribed to the fact that

patients from the lower socioeconomic and predominantly black African communities often present much later in the course of infection than their counterparts from the higher socioeconomic communities; consequently, HuCV excretion could be on the wane and below the level of detectability by direct EM. In addition, specimens from non-hospitalised patients may not have been stored and transported under optimal conditions, resulting in partial degradation of the HuCVs. The aetiological role of the HuCVs in the associated sporadic gastroenteritis is, however, difficult to establish. In ten (23%) of the HuCV-positive patients, for whom additional diagnostic data were available, no other potential pathogens were identified, while in another nine (21%), concomitant rotavirus, adenovirus, or parasitic infections were evident. The clinical significance of the HuCV infections, especially in the hospitalised patients, is also difficult to assess in view of the high incidence of rotavirus infections, which are known to be a significant cause of morbidity and mortality [Bishop, 1994].

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REFERENCES

Bishop RF (1994): Natural history of human rotavirus infections. In Kapikian AZ (ed): "Viral Infections of the Gastrointestinal Tract." New York: Marcel Dekker, pp 131–167.

Cubitt WD (1987): The candidate caliciviruses. In Bock G, Whelan J (eds): "Novel Diarrhoea Viruses." Ciba Foundation Symposium 128. Chichester: John Wiley & Sons, pp 126–143.

Cubitt WD (1989): Diagnosis, occurrence and clinical significance of the human "candidate" caliciviruses. Progress in Medical Virology 36:103–119.

Cubitt WD, Jiang X (1996): Study on occurrence of human calicivirus (Mexico strain) as cause of sporadic cases and outbreaks of calicivirus-associated diarrhoea in the United Kingdom, 1983–1995. Journal of Medical Virology 48:273–277.

Cubitt WD, Jiang XJ, Wang J, Estes MK (1994): Sequence similarity of human caliciviruses and small round structured viruses. Journal of Medical Virology 43:252–258.

Dinulos MB, Matson DO (1994): Recent developments with human caliciviruses. Pediatric Infectious Disease Journal 13:998–1003.

Graham DY, Jiang X, Tanaka T, Opekun AR, Madore HP, Estes MK (1994): Norwalk virus infection of volunteers: New insights based on improved assays. Journal of Infectious Diseases 170:34–43.

Green J, Norcott JP, Lewis D, Arnold C, Brown WG (1993): Norwalk-like viruses: Demonstration of genomic diversity by polymerase chain reaction. Journal of Clinical Microbiology 31:3007–3012.

Green J, Hale AD, Brown DWG (1995a): Recent developments in the detection and characterisation of small round structured viruses. PHLS Microbiology Digest 12:219–222.

Green J, Gallimore CJ, Norcott JP, Lewis D, Brown DWG (1995b): Broadly reactive reverse transcriptase polymerase chain reaction for the diagnosis of SRSV-associated gastroenteritis. Journal of Medical Virology 47:392–398.

Green SM, Dingle KE, Lambden PR, Caul EO, Ashley CR, Clarke IN (1994): Human enteric *Caliciviridae:* A new prevalent small round-structured virus group defined by RNA-dependent RNA polymerase and capsid diversity. Journal of General Virology 75: 1883–1888.

Herwaldt BL, Lew JF, Moe CL, Lewis DC, Humphrey CD, Monroe SS, Pon EW, Glass RI (1994): Characterization of a variant strain of Norwalk virus from a food-borne outbreak of gastroenteritis on a cruise ship in Hawaii. Journal of Clinical Microbiology 32:861–866.

Jiang X, Graham DY, Wang K, Estes MK (1990): Norwalk virus genome cloning and characterization. Science 250:1580–1583.

Jiang X, Wang J, Graham DY, Estes MK (1992): Detection of Norwalk virus in stool by polymerase chain reaction. Journal of Clinical Microbiology 30:2529–2534.

Jiang X, Matson DO, Velaquez FR, Calva JJ, Zhong WM, Hu J, Ruiz-Palacios GM, Pickering LK (1995a): Study of Norwalk-related viruses in Mexican children. Journal of Medical Virology 47:309–316.

Jiang X, Matson DO, Ruiz-Palacios GM, Hu J, Treanor J, Pickering LK (1995b): Expression, self-assembly, and antigenicity of a Snow Mountain agent-like calicivirus capsid protein. Journal of Clinical Microbiology 33:1452–1455.

Jiang X, Wang JX, Estes MK (1995c): Characterization of SRSVs using RT-PCR and a new antigen ELISA. Archives of Virology 140: 363–374.

Jiang X, Cubitt D, Hu J, Dai X, Treanor J, Matson DO, Pickering LK (1995d): Development of an ELISA to detect MX virus, a human calicivirus in the Snow Mountain agent genogroup. Journal of General Virology 76:2739–2747.

Kapikian AZ (1994): Norwalk and Norwalk-like viruses. In Kapikian AZ (ed): "Viral Infections of the Gastrointestinal Tract." New York: Marcel Dekker, pp 471–518.

Lambden PR, Caul EO, Ashley CR, Clarke IN (1993): Sequence and genome organization of a human small round structured (Norwalk-like) virus. Science 259:516–518.

Lew JF, Kapikian AZ, Valdesuso J, Green KY (1994): Molecular characterisation of Hawaii virus and other Norwalk-like viruses: Evidence for genetic polymorphism among human caliciviruses. Journal of Infectious Diseases 170:535–542.

Lewis DC, Green J, Eglin R, Brown DW (1993): Norwalk-like viruses in the UK. A survey of "serotype" prevalence over a 3 year period. Glasgow, Scotland: Abstracts of the IXth International Congress of Virology (ICV), p 137.

Liu BL, Clarke IN, Caul EO, Lambden PR (1995): Human enteric caliciviruses have a unique genome structure and are distinct from the Norwalk-like viruses. Archives of Virology 140:1345–1356.

Matson DO, Zhong WM, Nakata S, Numata K, Jiang X, Pickering LK, Chiba S, Estes MK (1995): Molecular characterization of a human calicivirus with sequence relationships closer to animal calicivirus than other known human caliciviruses. Journal of Medical Virology 45:215–222.

Moe CL, Gentsch J, Ando T, Grohmann G, Monroe SS, Jiang X, Wang J, Estes MK, Seto Y, Humphrey C, Stine S, Glass R (1994): Application of PCR to detect Norwalk virus in fecal specimens from outbreaks of gastroenteritis. Journal of Clinical Microbiology 32: 642–648.

Monroe SS, Glass RI, Noah N, Flewett TH, Caul EO, Ashton CI, Curry A, Field AM, Madeley R, Pead PJ (1991): Electron microscopic reporting of gastrointestinal viruses in the United Kingdom. Journal of Medical Virology 33:193–198.

Norcott JP, Green J, Lewis D, Estes MK, Barlow KL, Brown DWG (1994): Genomic diversity of small round structured viruses in the United Kingdom. Journal of Medical Virology 44:280–286.

Numata K, Nakata S, Jiang X, Estes MK, Chiba S (1994): Epidemiological study of Norwalk virus infections in Japan and Southeast Asia by enzyme-linked immunosorbent assays with Norwalk virus capsid protein produced by the baculovirus expression system. Journal of Clinical Microbiology 32:121–126.

Parker SP, Cubitt WD, Jiang X (1995): Enzyme immunoassay using baculovirus-expressed human calicivirus (Mexico) for the mea-

surement of IgG responses and determining its seroprevalence in London, UK. Journal of Medical Virology 46:194–200.

Steele AD, Phillips J, Peenze I, Jiang XI (1995a): Identification and molecular characterization of a Snow Mountain agent-like virus associated with paediatric gastroenteritis in South Africa. Johannesburg, South Africa: Abstracts of the Vth International Congress on the Impact of Viral Diseases in the Developing World, p 125.

Steele AD, Smit TK, Peenze I, Jiang XI, Estes MK (1995b): Epidemiology of Norwalk virus infections in Ga-Rankuwa, South Africa. Johannesburg, South Africa: Abstracts of the Vth International Congress on the Impact of Viral Diseases in the Developing World, p 126.

Taylor MB, Schildhauer CI, Parker S, Grabow WOK, Jiang X, Estes MK, Cubitt WD (1993): Two successive outbreaks of SRSV-associated gastroenteritis in South Africa. Journal of Medical Virology 41:18–23.

Taylor MB, Parker S, Grabow WOK, Cubitt WD (1996): An epidemiological investigation of Norwalk virus infection in South Africa. Epidemiology and Infection 116:203–206.

Tiemessen CT, Wegerhoff FO, Erasmus MJ, Kidd AH (1989): Infection by enteric adenoviruses, rotaviruses, and other agents in a rural African environment. Journal of Medical Virology 28:176–182.

Treanor JJ, Jiang X, Madore P, Estes MK (1993): Subclass specific serum antibody responses to recombinant Norwalk capsid antigen (rNV) in adults infected with Norwalk, Snow Mountain, or Hawaii viruses. Journal of Clinical Microbiology 31:1630–1634.

Wang J, Jiang X, Madore HP, Gray J, Desselberger U, Ando T, Seto Y, Oishi I, Lew JF, Green KY, Estes MK (1994): Sequence diversity of small round structured viruses. Journal of Virology 68:5982–5990.

Wolfaardt M, Taylor MB, Grabow WOK, Cubitt WD, Jiang X (1995): Molecular characterisation of small round structured viruses associated with gastroenteritis in South Africa. Journal of Medical Virology 47:386–391.